Glaser #12

lee-side lamina deposition; secondly, over-stepping sets with diastemic or other sharp boundaries, built by lee-side lamina deposition in excess of stoss-side erosion; and thirdly, the over-stepping drift profiles of Sorby[11] with their gradational boundaries due to lamina deposition on both lee- and stoss-sides. The mark-profiles are deposited under low relative rates and the drift-profiles under high relative rates of sedimentation. For reasons which are still not clear, fossil ripple-mark and ripple-drift are common while fossil dune-mark and dune-drift are rare or absent in Nature.

Walker[12] described a special form of ripple-drift, his type 3, which is often closely associated with turbidites. Mud, silt and sand grade particles are found segregated to some extent in these structures, the more muddy fraction [illegible] the lower lee-slopes and the sandy fraction on the crests and stoss-sides. I believe that non-random eddy [illegible] are necessary to build these, just as they are necessary to build other cross-stratification structures; but that their association with turbidites and the only partial segregation of a considerable range of grade sizes suggest that they were built by a current carrying a small [illegible] of suspended material. This load was [illegible] limit beyond which any increase [illegible] saltation and prohibit cross-lamina [illegible].

[illegible] ripple-marks consist of 'back-to-[illegible]' lamina, built by eddies set up [illegible] each mark by an oscillating current [illegible] demonstrated, and which has recently [illegible] as a 'figure-of-eight' current [illegible] therefore built by the same basic [illegible] symmetrical ripple-marks.

[illegible] stratification occurs in the upper [illegible] Thompson[14], and subaerially in the [illegible] described by Bagnold[15]. It consists [illegible] of low-angle cross-strata, less than [illegible] horizontal and with overlap and over-[illegible] relationships between sets of slightly different cross-[illegible] dips. This geometry together with certain textural characteristics distinguishes these structures from other cross-stratification and they may perhaps be collectively referred to as plinth-sets. Their distinctive nature appears to be due to deposition on slopes opposing the direction of sediment transport and it is interesting to compare the low-angle [illegible] of the stoss-side laminæ of ripple-drift.

Gradation between toe-sets or tangential and angular cross-stratification, and also the presence or absence of irregular [illegible] bases due to slight erosion, are, according to [illegible] experimenters, features related to variations in the activity of lee-side eddies. These variations, alternations of cross-strata lithologies and other forms of sub-sets, are here regarded as minor characters [illegible] as bases for the erection of cross-stratification sub-types. The special form of ripple-drift with muddy lee sides, described by Walker[12], should also be regarded as a [illegible] of ripple-drift.

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The terms ripple [illegible] mark [illegible] and drift, straight, [illegible] and rhomboid, [illegible] going paragraphs and in the papers specif[illegible] provide adequate well-established unit[illegible] a logical cross-stratification terminology can be [illegible]. This is listed in Table 1 with the better-known synonyms.

Table 1. Cross-Stratification Terminology

Present article	Allen, 1963 (ref. 4)	Some pre-1963 equivalents
Symmetrical ripple-marks	—	Structure of wave ripple-mark
Straight ripple-marks	—	Structure of [illegible] ripples
Linguoid ripple-marks	—	Structure of linguoid ripples
Straight ripple-sets	Mu	
Linguoid ripple-sets	Nu	Micro-cross-lamination[16]
Rhomboid ripple-sets		Structure developed from rhomboid [illegible]
Straight ripple-drift	Lambda	Ripple-drift, part of [illegible]
Linguoid ripple-drift	Kappa	Ripple-drift, part of [illegible]
Straight dune-sets	Omikron	[illegible]
Linguoid dune-sets	Pi	[illegible]
Bar-sets	Alpha (part of)	[illegible]
Plinth-sets	Xi	[illegible]

The word 'asymmetrical' is omitted [illegible] recommended here, for the sake of [illegible] word terms refer to three-dimensional [illegible] the exception of the first listed term [illegible] that they may be shortened to the appropri[illegible] terms when only two-dimensional [illegible] are described.

Cross-stratification may fill [illegible] excavations [illegible] shapes, many if not all of which [illegible] distinct from the deposition of [illegible] stratifications and excavations [illegible] as separate structures[18].

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EFFECT OF A MAGNETIC FIELD ON CARBOXYDISMUTASE

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INVESTIGATIONS of the effect of a magnetic field on biological systems are numerous. Leusden[1] working with coliform organisms and *Staphylococci*, and Jennison[2] with 20 species of bacteria, yeasts and moulds, found no effect of a magnetic field on their growth. Margou and Marigault[3] reported that a magnetic field can retard growth of tumours on a strain of *Pelargonium zonale*. Recently it has been reported[4] that a magnetic field does not affect the fission rate of bacteria but it has a mortal effect, probably due to paramagnetic phenomena. During incubation, a mutant strain develops resistance to magnetic fields[5]. The responses of the planarian, *Dugesia dorotocephala*, to very weak horizontal magnetic fields[6] as well as of the mud-snail, *Nassarius obsoletus*, to magnetism with the strength of geomagnetism have recently been reported, opening the fascinating question of geomagnetic effect on all living matter. Beischer[7], extending the work of Perakis, has examined the effect of strong magnetic fields on the fertilization and development of sea-urchin eggs and he reported that the uniform field seems to have beneficial effect on the development while the non-uniform field reduce the survival rate. The effect of [illegible] 6,000 oersteds on the growth of mice has recently been reported[8].

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Commoner *et al.*[10] have demonstrated *in vitro* the presence of unpaired electrons in enzymatic reactions; therefore one would expect an influence of magnetism on the reaction rate, since paramagnetic molecules are present. Beischer[7] has examined the effect of uniform and non-uniform magnetic fields on alcohol dehydrogenase and peroxidase and he was not able to demonstrate any specific effect. Sister Smith and Cook[11] have recently shown that magnetic fields of at least 5,000 gauss raise the enzymatic activity of trypsin.

The work reported here is the study of the effect of a magnetic field of about 20,000 gauss on the activity of carboxydismutase, the enzyme which catalyses the primary carboxylation reaction in photosynthesis, that is, the reaction:

$$\text{ribulose-1,5-diphosphate} + HCO_3^- \xrightarrow{\text{carboxydismutase}} \text{3-phosphoglycerate}$$

Carboxydismutase was prepared from spinach chloroplasts (*Spinacea oleracea*) as described previously[12,13]. The enzyme was dissolved in potassium phosphate buffer pH 7·4 and kept at 0° C. The protein content of the solution was determined on the basis of dry weight. The purity of the enzyme was estimated to be approximately 70 per cent.

In all our experiments, a double-wall tube containing the enzyme solution was placed between the tapered pole caps (off) Spectromagnetic Industries model TC-200-8 4-in. electromagnet (Spectromagnetic Industries, Hayward, California). The average field strength was 20,000 gauss. Another similar tube, serving as control, was placed outside the magnet. Both tubes as well as the poles of the magnet were cooled by circulating cold water (4° C) (Fig. 1), and were covered with a piece of black fabric in order to eliminate any effect due to light. 0·5 ml. of enzyme solution in phosphate or *tris* buffer pH 8·0 were used in each experiment, and at different intervals of time (30 min–6 days) samples were taken and checked for enzymatic activity.

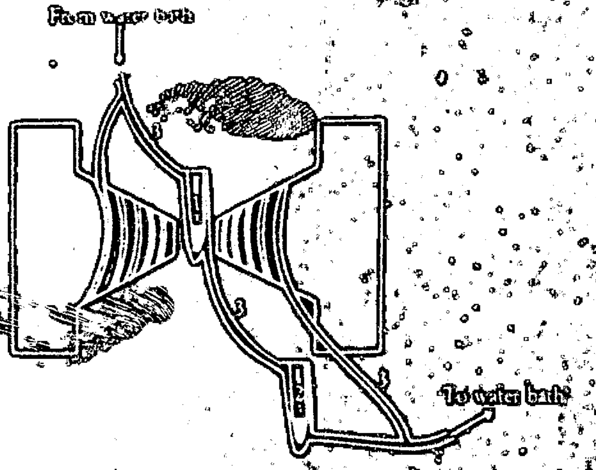

Fig. 1. Magnet with sample in position. 1, Sample in double-wall tube between the pole caps of the electromagnet, in a field strength of 20,000 gauss; 2, control tube; 3, thermostatically controlled water-circulating device

The carboxydismutase assay was the following: enzyme solution, 1 M *tris* buffer pH 8·0, magnesium chloride, and bicarbonate labelled with carbon-14 were pre-incubated for 10 min at 0° C, then ribulose-1,5-diphosphate (RuDP) solution was added and the mixture was incubated for 15 min at 22° C. The reaction was stopped by the addition of acetic acid solution and the 3-phosphoglyceric acid radioactivity was determined as acid stable radioactivity. An aliquot of the mixture was spread out on a thin aluminium planchet with the aid of a *micropipetto*, dried under an infra-red lamp and counted. An automatic gas flow counter equipped with a Geiger-Müller tube with a 'Micromil' window was used for precise counting.

More than twenty experiments were performed and the results of all of them showed a pronounced activating effect of 14–20 per cent on carboxydismutase. Table 1 shows the difference in activity between the enzyme exposed to the magnetic field and of that serving as control of a representative set of experiments. The loss in activity as a function of time is due to denaturation of the enzyme which occurs; however, this inactivation is more pronounced in the case of the non-exposed to the magnetic-field enzyme than in that of the exposed.

Table 1. Effect of Magnetic Field on the Activity of Carboxydismutase

Hours in the magnetic field	Hours out of the magnetic field	d.p.m. mg protein × 10	
1	—	1·66	
—	1		1·50
2	—	1·93	
—	2		1·70
48	—	1·69	
—	48		1·44
192	—	1·37	
—	192		1·00

Experimental conditions: Each reaction mixture contained (μmol.): *tris* pH 8·0, 25; enzyme, 40 μg (dry weight); $NaH^{14}CO_3$ (specific activity 21 μc./μM), 2; RuDP, 0·09. Final volume 800 μl. Pre-incubation (without RuDP), 10 min at 0° C. Incubation following RuDP addition, 15 min at 22° C. Magnetic-field strength, 20,000 gauss. Temperature of the enzyme mixture at the time of its exposure to magnetic field, 4° C.

It was also found that there was not any difference in the results whether the pre-incubation and incubation of the enzyme with the other components of the reaction mixture were done inside or outside the magnetic field.

To eliminate any questions as to the validity of the control, some experiments were performed where the same enzyme solution served as both control and test sample. In these experiments enzyme solutions in *tris* buffer pH 8·0 were placed in the magnet which was turned on and off every 24 h for 6 days, and at different intervals of time samples were taken and checked for activity. The average results of three experiments are shown in Fig. 2. The activity of carboxydismutase is increased considerably as the magnet is turned on, while as soon as the magnet is turned off the activity decreases.

The activating effect of a magnetic field on enzymes is quite a new discovery and opens up a fascinating field of further studies. The effect seems to be the inverse of denaturation. The ultra-violet spectrum of trypsin was found by Sister Smith and Cook to change considerably after exposure to the magnetic field and the change was exactly the inverse of that of the spectrum of the ultra-violet irradiated enzyme; in other words the qualitative change produced by ultra-violet irradiation was the elevation of the 220–230 mμ spectrum, while that produced by the magnetic field was the depression of the spectrum in the same region.

Some experiments were also performed in our laboratory, in which the enzyme was partially or completely inactiv-

Fig. 2. Effect of a magnetic field on the activity of carboxydismutase. The same enzyme solution was serving as both control and test sample and only the magnet was turned on and off. All conditions and components were as in experiment of Table 1

ated by ultra-violet light prior to exposure to the magnetic field, and the results showed that carboxydismutase previously irradiated by ultra-violet light could become reactivated under the influence of the magnetic field. Therefore, denaturation of carboxydismutase by the ultra-violet light cannot be overcome and reversed by the magnetic fields. However, the change produced by the magnetic field is a reversible one as shown in Fig. 2. Sister Smith and Cook interpreted the activating effect of the magnetic field as being due to an increase in hydrogen bonding and consequently in helicity of the polypeptide backbone of the enzyme. If the influence of the magnetic field is the increase in its helicity or in general in its symmetrical configuration, then this action can only take place as far as the reaction:

$$\text{native carboxydismutase} \underset{}{\overset{\text{magnetic field}}{\rightleftharpoons}} \text{activated (helical) carboxydismutase}$$

is concerned. In the case of the denatured enzyme the tertiary structure is quite distorted to form randomly coiled parts and the magnetic field cannot affect its renaturation. The increase in helical structure and hydrogen bonding stabilizes the protein against denaturation; thus the enzyme is less apt to lose its activity. Notice in this respect that the enzyme after prolonged exposure to the magnetic field is not denatured so fast as the control. Therefore, one can conclude that, if this is the true activating mechanism of the magnetic-field influence on an enzyme, the catalytically active part of the surface of the enzyme may include side-chains of amino-acids well separated in the sequence of the polypeptide chain, which are brought together under the influence of the magnetic field, by folding back of the main chain.

Recently, the specific enzymatic activity of adenosine triphosphatase of skeletal myosin A was found to be increased by ethylene glycol at a concentration of 50 volumes per 100 ml. (ref. 14); optical rotatory dispersion examinations of the activated myosin showed that a pronounced unfolding of the helical structure occurs. In this case, quite a different activating mechanism takes place, and raises the question whether the effect of magnetic field is due to a hyperfolding (folding in excess of native form) or unfolding mechanism. Optical rotatory dispersion measurements of the activated and the native protein will give us an answer to that question.

The effect of electrostatic field on the activity of carboxydismutase was also investigated. In our preliminary experiments it was found to affect the activity of the enzyme in a way similar to that of the magnetic field.

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TRANSGLUCOSYLASE ACTIVITY OF FUNGAL LAMINARINASES

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DURING investigations of laminarin degradation we have observed that many fungal enzyme preparations exhibited transglucosylation activity. It was important to estimate the extent of this, because the multicomponent nature of fungal laminarinases and the structure of laminarin were being investigated. Laminarin, a food reserve found in the fronds of sub-littoral brown algae, especially species of *Laminaria*, is a β-D-1,3 glucan containing occasional β-1,6 linkages which may constitute branch points in the molecule. Cellobiose has been detected frequently in enzyme digests of laminarin[1]. As considered here transglucosylation is a synthesis of oligosaccharides from simple, low-energy substrates without the participation of phosphorylated sugars. It may be represented thus:

$$\text{glucosyl-}X + \text{enzyme-H} \rightarrow \text{glucosyl enzyme} + X\text{H}$$
$$\text{glucosyl enzyme} + R\,\text{OH} \rightarrow \text{glucosyl-O}\,R + \text{enzyme-H}$$

This must be distinguished from condensation reactions involving glucose with the production of disaccharides. Condensations are usually slow and require large substrate concentrations.

Fungi degrade laminarin by extra-cellular, multi-component enzyme systems; the laminarinase complex and the isolation and purification of these are described elsewhere[1,2]. Three main types of laminarinase complex were evident, predominantly exo-hydrolytic, predominantly endo-hydrolytic and intermediate, representative systems, that is, from *Trichoderma viride*, *Rhizopus nodosus* and *Myrothecium verrucaria*, were used in all experiments.

Digests consisted of substrate, 10 per cent (w/v) in 2 ml. acetate buffer (0·1 M, pH 5·8) (ref. 3), incubated with 50 u of laminarinase at 37° C (1 u of activity ≡ production of 1 mg reducing sugar, as glucose, from a 0·5 per cent (w/v) solution of laminarin in 30 min)[1]. Substrate solutions were sterilized by Seitz filtering and a few drops of toluene were added to prevent bacterial growth. Transglucosylation was followed by paper chromatographic analysis of the digests, the solvent system n-butanol-pyridine-water-benzene (5 : 3 : 3 : 1) (ref. 4) giving excellent separations of nearly all oligosaccharides. Other systems were used for specific problems and for confirming identifications. Reference compounds were gifts from various laboratories, obtained commercially, or prepared by one of us (A. T. B.). The purity of these compounds was checked chromatographically. The logarithmic partition function α, defined as $\log \frac{1-Rg}{Rg}$, is a linear function of molecular size for any series of oligosaccharides (following ref. 5). This relationship has been used as an additional means of identifying synthesized sugars.

Preliminary experiments investigated the extent of glucose condensation, digests being analysed over a 720-h incubation period. Significant condensations occurred only in the presence of large substrate concentrations, namely, 50 per cent (w/v). Three disaccharides—gentibiose, cellobiose and laminaribiose—and three trisaccharides—cellotriose, 3-O-β-(cellobiosyl)-D-glucose, and 3-O-β-(gentiobiosyl)-D-glucose—were synthesized, though most condensation products were degraded after 720 h.